Proliferative Epithelial Lesions of the Urinary Bladder in Cynomolgus Monkeys (Macaca fascicularis) Infected with Schistosoma intercalatum

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SUMMARY

Five cynomolgus monkeys (Macaca fascicularis) were infected with Schistosoma intercalatum, a helminth that is morphologically similar to Schistosoma haematobium. Infections were readily established and remained active until the monkeys were sacrificed 21 to 84 weeks after exposure. Although the schistosomes were located predominantly in mesenteric and hepatic portal venules, schistosome eggs were found in the bladders of 3 monkeys. Nodules of atypical epithelial cells interpreted as superficially infiltrating undifferentiated bladder carcinomas were found in one monkey 23 weeks after infection. These sessile tumors differ strikingly from the well-differentiated, papillary transitional cell tumors previously reported from several species of experimental animals infected with S. haematobium. The tumors are also dissimilar to the squamous cell bladder tumors associated with S. haematobium infection in man but may nonetheless be useful for investigations of schistosomal bladder cancer.

INTRODUCTION

The significance of Schistosoma haematobium as a pathogen for man is well recognized in the endemic areas of Africa and the Middle East. Significant pathology has also been produced in numerous experimental hosts (2, 6, 9, 15, 20). Schistosoma intercalatum, a close relative of S. haematobium, occurring in the peoples of Central and West Africa (3, 4, 18), received little attention until recent years. In our studies on the biology of schistosomes, emphasizing development of models and the evaluation of the carcinogenic potential of different species, we have exposed many mammals to the terminal spine schistosomes (2, 9, 10, 12, 13). Proliferative epithelial lesions of the bladder have been demonstrated in several primate species infected with S. haematobium (7, 8).

This study is concerned with the host-parasite relationships and the pathology in cynomolgus monkeys subsequent to exposure to moderate numbers of S. intercalatum.

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MATERIALS AND METHODS

Five cynomolgus monkeys (M. fascicularis Raffles 1821) were procured from an animal importer (Primate Imports, Port Washington, N. Y.) and were held for 3 years prior to use in this study.

The original stock of S. intercalatum (Cameroon strain) was kindly provided by Dr. C. A. Wright, British Museum, and has been maintained in the laboratory at San Antonio for approximately 3 years. Cercariae of S. intercalatum were pooled from 24 to 33 infected Bulinus wrighti (Southern Arabia) that had been exposed to miracidia hatched from eggs obtained from the large intestine of hamsters. Hosts were lightly anesthetized (phencyclidine hydrochloride), and the abdominal hair was removed by clipping, after which the skin was well cleansed with water prior to exposure to counted numbers of cercariae on coverslips. Fecal and urine samples were examined for schistosome eggs at intervals of 1 to 2 weeks. The Stoll technique (16) was used for fecal egg counts based on 24-hr samples, and the sediment of centrifuged samples of urine was examined. At sacrifice, organs were examined for presence of schistosomes. The perfusion technique (14) was used to remove parasites when feasible, and the remainder was extracted manually under the dissecting microscope.

At sacrifice, the urinary bladder was filled with 10% buffered formalin. Eggs in tissues were counted following digestion in 4% KOH (1). Tissues from the urogenital system, fixed in formalin, were digested at 56° for 18 hr while unfixed samples from all other organs were digested at 37° for 12 to 14 hr. Samples for histopathology were sectioned at 4 to 6 μm and stained with hematoxylin and eosin.

RESULTS

Parasitological Observations. Pertinent data on host-parasite relationships, including the number and distribution of schistosomes at sacrifice, are given in Table 1. Of the 1000 cercariae applied, 2.7 to 36.1% were recovered as adult worms at necropsy. There was a dominance of male schistosomes even though a minimum of 24 snails was used as the source of cercariae. Only 1 monkey (R-5) harbored parasites in association with the urinary bladder. Eggs first appeared in the feces 53 to 56 days after exposure. No eggs were found in the urine.
In general, the number of eggs in tissues was low and there was a broad range in the total number of eggs and in the number of eggs per worm pair recovered (Table 2). A large proportion of eggs occurred in the tissues of the large intestine of 4 monkeys, and 3 of 5 animals had low numbers of eggs in the bladder and ureters.

**Pathological Observations.** Three of 5 monkeys had macroscopic bladder lesions. In Monkeys R-4 and R-5, 10 to 20% of the bladder surface was covered by firm, elevated tan nodules measuring 0.2 to 0.4 cm in diameter and were elevated about 0.1 cm above the bladder surface (Fig. 1). Occasional submucosal nodules were seen in Monkey R-8. Bladder nodules showed 3 distinct types of histology. Roughly one-half of the nodules were caused by submucosal inflammatory infiltrates. Some of these contained schistosome eggs, but many did not. Most of the remaining bladder nodules were areas of cystitis glandularis in which the epithelium was transitional and showed only minimal atypia. Three of the nodules examined in Monkey R-4 showed nests and interlacing strands of undifferentiated epithelial cells with large, pleomorphic, hyperchromatic nuclei and abundant eosinophilic cytoplasm (Figs. 2 to 4). Tumor cells extended singly and in small strands into the submucosal inflammatory infiltrate (Figs. 3 and 4), which was composed of eosinophils, plasma cells, and lymphocytes. Tumor did not extend deeply into submucosal connective tissue or into bladder muscle. No metastases were present. Small numbers of squamous cells were present at the margin of the lesions, but no tumor cells could be clearly identified as squamous. No papillary element was present. Three apparently independent tumors were sectioned, 1 in the apex and 2 in the trigone. The tumors were topographically related to sites of egg deposition and inflammation, and all were adjacent to areas of cystitis glandularis. In 1 lesion in Monkey R-5, the cystitis glandularis was more extensive than that in other monkeys (Fig. 5) and moderate atypia was present (Fig. 6). This lesion thus had histological features intermediate between the usual areas

### Table 1

**S. intercalatum infection in M. fascicularis exposed to 1000 each (worm recovery and distribution)**

<table>
<thead>
<tr>
<th>Host</th>
<th>Sex</th>
<th>Duration of infection (wk)</th>
<th>Liver + Hepatic portal veins</th>
<th>Small intestine</th>
<th>Pancreas</th>
<th>Large intestine</th>
<th>Urinary bladder</th>
<th>Extra</th>
<th>Total no.</th>
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</thead>
<tbody>
<tr>
<td>R-2</td>
<td>M</td>
<td>21</td>
<td>56</td>
<td>34</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>28</td>
<td>61</td>
</tr>
<tr>
<td>R-8</td>
<td>M</td>
<td>21</td>
<td>22</td>
<td>59</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>R-4</td>
<td>M</td>
<td>23</td>
<td>26</td>
<td>24</td>
<td>3</td>
<td>47</td>
<td>0</td>
<td>149</td>
<td>63</td>
</tr>
<tr>
<td>R-5</td>
<td>M</td>
<td>25</td>
<td>5</td>
<td>23</td>
<td>0</td>
<td>71</td>
<td>1</td>
<td>98</td>
<td>5</td>
</tr>
<tr>
<td>R-6</td>
<td>M</td>
<td>84</td>
<td>6</td>
<td>21</td>
<td>0</td>
<td>73</td>
<td>0</td>
<td>52</td>
<td>13</td>
</tr>
</tbody>
</table>

* Values given as nearest whole number.

### Table 2

**S. intercalatum infection in M. fascicularis exposed to 1000 cercariae each (egg distribution and number in the tissues)**

<table>
<thead>
<tr>
<th>Host</th>
<th>Duration of infection (wk)</th>
<th>Lungs</th>
<th>Liver</th>
<th>Stomach</th>
<th>Small intestine</th>
<th>Pancreas</th>
<th>Colon</th>
<th>Mesentery</th>
<th>Genital</th>
<th>Bladder-ureters</th>
<th>Total eggs recovered (in 1000's)</th>
<th>Eggs/pair of worms</th>
<th>Eggs/g bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-2</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>89</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
<td>169</td>
<td>0</td>
</tr>
<tr>
<td>R-8</td>
<td>21</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>39</td>
<td>31</td>
<td>4</td>
<td>0.7</td>
<td>9.2</td>
<td>710</td>
<td>5</td>
</tr>
<tr>
<td>R-4</td>
<td>23</td>
<td>0</td>
<td>6</td>
<td>Ns</td>
<td>21</td>
<td>1</td>
<td>68</td>
<td>4</td>
<td>NS</td>
<td>0.1</td>
<td>1009.9</td>
<td>7382</td>
<td>56</td>
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<tr>
<td>R-5</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>0.1</td>
<td>10</td>
<td>1</td>
<td>79</td>
<td>8</td>
<td>0.1</td>
<td>0.4</td>
<td>446.9</td>
<td>4581</td>
<td>249</td>
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<tr>
<td>R-6</td>
<td>84</td>
<td>1</td>
<td>15</td>
<td>0</td>
<td>58</td>
<td>0.5</td>
<td>16</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>107.5</td>
<td>2066</td>
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</tbody>
</table>

* NS, not significant (<0.1%).

### Table 3

**Record of patency and number of eggs passed with 24-hr fecal sample**

<table>
<thead>
<tr>
<th>Host</th>
<th>Duration of infection (wk)</th>
<th>Patency (days)*</th>
<th>Eggs passed on (day of patency)</th>
<th>Maximum eggs passed (days postpatency)</th>
<th>Eggs passed immediately prior to necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-2</td>
<td>21</td>
<td>56</td>
<td>1,590</td>
<td>12,654 (21)</td>
<td>0</td>
</tr>
<tr>
<td>R-8</td>
<td>21</td>
<td>56</td>
<td>20,002</td>
<td>20,002 (0)</td>
<td>0</td>
</tr>
<tr>
<td>R-4</td>
<td>24</td>
<td>54</td>
<td>885</td>
<td>25,432 (37)</td>
<td>984</td>
</tr>
<tr>
<td>R-5</td>
<td>24</td>
<td>53</td>
<td>3,283</td>
<td>32,940 (42)</td>
<td>3,630</td>
</tr>
<tr>
<td>R-6</td>
<td>84</td>
<td>53</td>
<td>1,443</td>
<td>28,196 (70)</td>
<td>2,929</td>
</tr>
</tbody>
</table>

* Days postexposure.
* Egg counts based upon Stoll technique.
* Numbers in parentheses, days postpatency.
* Samples taken 2 to 4 days prior to necropsy.
of cystitis glandularis and the superficially infiltrating tumors noted in Monkey R-4.

Small numbers of schistosome eggs were present in the ureters and were associated with focal inflammation, slight thickening, and focal squamous metaplasia of the ureteral epithelium. The colon contained moderate numbers of eggs in the mucosa and submucosa with granulomas around the latter. Marked portal infiltrates of polymorphonuclear leukocytes, plasma cells, and lymphocytes were present in the liver, as were moderate numbers of circumoval granulomas. The latter were composed of polymorphonuclear neutrophils and mononuclear inflammatory cells and showed no fibrosis. Focal peribiliary fibrosis was not apparently related to the infection. The lungs and pancreas contained occasional granulomas. The small intestine, kidneys, and reproductive organs were unremarkable (Table 3).

**DISCUSSION**

*S. intercalatum* has been recognized as a parasite of man in isolated regions of Africa for several decades. Infection is usually confined to the mesenteric circulation, so that eggs are found in the liver, intestine, and feces but seldom in the urine (18). *S. intercalatum* infections have been studied little experimentally. Wright et al. (21) described the susceptibility of several domestic animals and nonhuman primates to infection by *S. intercalatum*. Taylor et al. (17) found eggs and circumoval granulomas in the bladders of baboons infected with *S. intercalatum*. We have not previously seen significant pathology in the bladder of several primate species examined (R. E. Kuntz, J. A. Moore, and T. C. Huang, unpublished observations).

We interpret the lesions in the bladder of Monkey R-4 as multicentric, superficially invasive carcinomas without clear differentiation as to cell type. Since the lesions did not metastasize or invade bladder muscle, the malignant character of these tumors has not been demonstrated biologically, and our interpretation rests entirely on morphological grounds. These sessile, undifferentiated lesions differ strikingly from the papillary transitional cell lesions of the bladder and ureters that we have noted in opossums and in several primate species infected with *S. haematobium* (7-9). In these lesions, cellular atypia was minimal and the lesions were primarily papillary and exophytic. Neither the lesions described here nor the papillary lesions are similar to the bladder tumors associated with *S. haematobium* infection in man (5). In view of the ability of bladder epithelium to differentiate in various ways (11) and the early nature of the lesions, this dissimilarity does not seriously detract from the potential usefulness of cynomolgus monkeys infected with *S. intercalatum* as a model system for study of schistosomal bladder cancer. More pertinent questions concern the reproducibility of the lesions, their progression with time, and the possibility of producing similar lesions with *S. haematobium*.

Cystitis and ureteritis glandularis are common in *S. haematobium* infection of the urinary tract in man (5) and experimental animals (2, 20). The topographic relation between the tumors in Monkey R-4 and the associated cystitis glandularis may simply reflect the dependence of each on local egg deposition.

**REFERENCES**

18. van Wijk, H. B. *Schistosoma intercalatum*-Infection in Mungo Department, Cameroon, 155 pp. Academisch Proefschrift, University of Amsterdam, 1975.
Fig. 1. Macroscopic lesions in urinary bladder of cynomolgus monkey (M. fascicularis) (R-4) 23 weeks postinfection with 1000 cercariae of S. intercalatum. Several nodules proved to be inflammatory, others showed cystitis glandularis, and 3 examined microscopically showed superficially invasive tumor.

Fig. 2. Nodule in the trigone of Monkey R-4. Atypical cystitis glandularis is present in this portion of the nodule. Normal bladder is seen at the extreme left. H & E, ×64.

Fig. 3. Another area from this same nodule shows infiltrating epithelial cells. H & E, ×165.

Fig. 4. Strands of tumor cells infiltrate the subepithelial connective tissue. Tumor cells have large nuclei with large nucleoli and show considerable pleomorphism (compare with Fig. 6). Apex of bladder (R-4). H & E, ×400.

Fig. 5. Nodule in the bladder of Monkey R-5 shows cystitis glandularis and moderate chronic inflammation. H & E, ×64.

Fig. 6. A higher magnification of a gland in Fig. 5. Epithelial cells show only slight atypia compared with the surface epithelium and much less atypia than the tumor cells in Fig. 4. H & E, ×400.
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